channels. None of them showed significant modulation, suggesting that channel activity, rather than expression level, is affected in this model.

MR activation specifically in endothelial cells is therefore associated with increased BP and altered vascular reactivity, in absence of renal collecting duct mediated MR effects. This may be related to ion channels remodeling.

J026
A ROLE FOR L-WNK1 IN CARDIOVASCULAR DEVELOPMENT AND VASOCONSTRICTION

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Mutations at the WNK1 gene cause Gordon syndrome, a rare inherited form of arterial hypertension. The gene encodes a short kidney-specific isoform and a complete long isoform, L-WNK1, expressed ubiquitously and notably within the vascular system.

Objective — The aim of our study was to investigate the vascular role of L-WNK1 in mice.

Method — We used mice bearing a constitutive homozygous inactivation of L-WNK1 (L-WNK1-/-) which die in utero before day 13 of gestation as well as adult healthy heterozygous (L-WNK1+/-) and wild-type (L-WNK1+/+) littermates. We isolated vessels from L-WNK1+/- and L-WNK1+/+ to perform in vitro pharmacological studies in a wire-myograph system and an arteriograph system.

Results — We first showed that L-WNK1-/- embryos present growth retardation and severe oedema. An abnormal vascular remodeling was observed at day 10.5 within the primary vascular network of L-WNK1-/- embryos as well as in the yolk sac, suggesting an important role of L-WNK1 in cardiovascular development. We next showed that vascular diameters of pressurized arteries as well as arterial blood flow velocities measured by echo-Doppler were comparable between L-WNK1+/- and L-WNK1+/+ mice. Endothelium-dependent dilatations induced either by acetylcholine or by flow were also comparable between both groups of mice. In contrast, phenylephrine-induced vasoconstrictions were significantly reduced in L-WNK1+/- mice compared to L-WNK1+/+ mice in thoracic aorta as well as in mesenteric arteries (27.45% (P=0.04) and 39.6% (P=0.0015) decrease at maximal concentration, respectively) whereas potassium chloride contractions remained comparable. Furthermore, myogenic tone in L-WNK1+/- mesenteric arteries was also significantly blunted when compared to L-WNK1+/+ mice (P<0.0001).

Conclusions — Our results evidenced for the first time a vascular role for L-WNK1 in pressure- and agonist- induced contractions.

J027
SERUM RESPONSE FACTOR INVOLVEMENT IN MYOGENIC TONE REGULATION IN RESISTANCE ARTERIES

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Serum Response Factor (SRF) has emerged as a dispensable transcription factor for cellular growth but an absolutely essential orchestrator of actin cytoskeleton and contractile homeostasis. SRF is a founding member of MADS domain-containing family of transcription factors and is present in most, if not all, species from animals to plants and fungus kingdoms. Signaling to SRF occurs principally through mitogen-activated protein kinase (MAPK) or RhoA pathways that converge on the nucleus to stimulate gene expression. In the microcirculation, diameter adjustments in response to change in intraluminal pressure or flow mainly depend on the integrity of the vascular cytoskeleton. We thus hypothesised that the deletion in SRF will affect the mechanotransduction in resistance arteries. To address the role of SRF, the tail caudal and mesenteric arteries from inducible SRF KO mice and their littermate control were mounted in an arteriograph. Contraction to stepwise increase in pressure (myogenic tone) and relaxation to progressive increases in intraluminal flow were determined in each group. The protein and gene expressions were quantificated by western blot and qPCR. SRF deficiency reduced myogenic tone in tail arteries (CT:16.3±3.2% of Passive Diameter (PD) versus KO:5.9±2.3% PD; P<0.05) and in mesenteric arteries (CT:26±2.3% PD versus KO:11±1.8% PD; P<0.05). No effect was observed on the flow mediated dilation and receptor dependant contraction induced by Phenylephrine, Angiotensine II and U46619. SRF alteration induced modifications of contraction signalling pathways as p38 MAP kinase, Myosin Light Chain Kinase, Myosin Light Chain, Src, Caveolin 1 or cytoskeleton proteins. Furthermore, actin polymerisation was also spoiled. This study reported for the first time that the SRF inhibition with an inducible KO significantly affected specifically the myogenic tone. This suggests that SRF is involved in the pressure mechanotransduction in resistance arteries. Due to the central role of myogenic tone in vascular disorders and organs autoregulation this finding opens new perspectives in the pathophysiology of the microcirculation and provides new therapeutic targets.

J028
NEW INSIGHTS INTO THE ROLE OF EPAC IN CARDIAC MYOCYTES SIGNALLING AND HYPERTROPHY

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Epac (Exchange protein directly activated by cAMP), a guanine exchange factor (GEF) for the Ras-like GTPases Rap, has been identified as a novel cAMP sensor, alongside of PKA. We have previously shown that Epac activation, through β-adrenergic receptor stimulation, induces cardiomyocyte hypertrophy (Morel et al, 2005, Metrich et al, 2008).

This work was intended to identify the component of the Epac signaling cascade leading to cardiac hypertrophy and in particular

the implication of the Epac—induced small G proteins and theirs networks.

In cardiomyocyte primary culture, we show that Epac activates the small G protein Ras, Rac and Rap. The Epac-induced hypertrophic effect is mediated by Ras and Rac activation. This Epac-induced activation of Ras is not influenced by Rac or Rap and is due to a Ca2+ release in response to phospholipase C and IP3 receptor activation. Moreover, we show that Ras mediates the Epac-induced activation of the pro-hypertrophic CaMKII/MEF2 and calcineurin/NFAT signalling pathways which are both necessary for hypertrophy. Epac has been initially identified as a GEF for the small G proteins Rap. Surprisingly, the Epac hypertrophic effect is independent of its classical effector Rap. The Epac-induced Rap1 signalling cascade involves PKCε translocation, which is a key actor of multiple cellular phosphorylations. This finding is in agreement with other Rap functions reported in the literature such as cell to cell communication and adhesion.

Altogether, these data present new insights into the Epac signaling network with both pro-hypertrophic and non-hypertrophic effects.

- Morel E, Marcantoni A, Gastineau M, Birkedal R, Rochais F, Garnier A, Lompré AM, Vandecasteele G, Lezoualc'h F. cAMP-binding protein Epac induces cardiomyocyte hypertrophy. Circ Res. 97 : 1296-304, 2005

- Métrich M, Lucas A, Gastineau M, Samuel JL, Heymes C, Morel E, Lezoualc'h F.

Epac mediates beta-adrenergic receptor-induced cardiomyocyte hypertrophy. Circ Res. 102 : 959-65, 2008

Vendredi 3 avril 2009, de 11h00 à 12h30

K — HORMONES, SYSTEME RENINE-ANGIOTENSINE

K001

IS PRIMARY ALDOSTERONISM A CHANNELOPATHY ?

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In mouse models the genetic deletion of TWIK-related acid-sensitive K (TASK)-1 and TASK-3 channels removes an important background K current that results in a marked depolarization of adrenal zona glomerulosa cell membrane potential, leading to the autonomous overproduction of aldosterone. The importance of TASK channel dysfunction in human primary aldosteronism (PAL) however, is uncertain, motivating their molecular analysis. We screened coding exons and flanking intronic sequences of KCNK3 and KCNK9 (genes coding for TASK 1 and TASK 3 respectively) in 825 PAL patients for germline DNA sequence variants. A total of 14 different coding sequence variants were found in 19 patients. The variants include 8 different synonymous mutations and 6 different missense variants. In silico predictions (PolyPhen, SIFT and Alamut) suggested the non-synonymous mutations to be potentially damaging. Analysis of mutated channel function by heterologous expression, however, revealed the missense mutations detected were non-functional. As somatic mutations may be involved in some cases of sporadic PAL, sequencing 90 patients for tumoral DNA sequence variants is underway. We are also investigating expression of TASK 1 and TASK 3 by in-situ hybridization and immunohistochemistry on adrenal tissue sections of 150 PAL patients who have undergone surgery.

K002

ENDOTHELIAL ESTROGEN RECEPTOR ALPHA MEDIATES THE ATHEROPROTECTIVE ACTION OF ESTRADIOL IN LDLR DEFICIENT MICE

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Background — Although estrogen administration to hysterectomized menopausal women did not prevent the occurence of myocardial infarction in a randomized controlled trial (WHI 2004), epidemiological studies suggest and experimental results clearly demonstrate a major atheroprotective action of estrogens. The goal of the present study was to identify the cellular target(s) accounting for the estradiol (E2) beneficial action on fatty streak development.

Methods and Results — We first confirmed the key role of estrogen receptor α (ERα) in atheroprotective effect of E2 as this action was completely abolished in mice deficient both in Low Density Lipoprotein receptor (LDLr) and in ERα. Comparison of LDLr-/- mice transplanted with either ERα+/+ or ERα-/- bone marrow showed that functional ERα in the hematopoietic lineage is not required for E2 atheroprotection. We then showed that ERα floxed mice (ERαflox/flox) bred with the Tie2-Cre mice on the LDLr-/- background had a complete inactivation of ERα both in bone marrow and in endothelial cells. Remarkably, in this mouse model, the E2 atheroprotective action was completely abolished.

Conclusions — Altogether, this is the first in vivo demonstration that endothelial ERα represents a key target of the atheroprotective effect of E2, whereas the hematopoietic ERα is dispensable for the protective action. Selective estrogen receptor modulators that mimic this endothelial action of E2 should now be considered in hormonal treatment as well as in atheroprotection.

K003

MODELISATION PHYSIOLOGIQUE INTEGRATIVE DE LA REGULATION DE LA PRESSION ARTERIELLE ET DU SYSTEME RENINE-ANGIOTENSINE CIRCULANT

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Introduction — Le SRA est impliqué dans l'homéostasie hydrosodée et la régulation de la pression artérielle (PA). L'objectif du projet Saphir (Thomas et al., Phil Trans R Soc A, 2008) est le développement d'un modèle multiniveaux du système cardiovasculaire rénal (CVR). Nous présentons ici la modélisation du SRA circulant, intégré au modèle circulatoire de Guyton.

Modelisation — Le modèle est implémenté sous Simulink© (Mathworks). Hypothèses : (i) la rénine est produite par l'appareil juxtaglomérulaire (JGA) et l'artériole afférente ; (ii) la production de rénine est inhibée par la pression de perfusion, le signal TGF